centration in animal sera, or to a strict species specificity to streptokinase.

The precise role of ascorbic acid in repair processes is not known. The possibility exists that Vit. C may be utilized by injured tissues for synthesis of collagen(20). Lemberg, *et al.*,(21) reported that coupled oxidation may occur between hemoglobin and ascorbic acid for the formation of choleglobin. Bourne (22) established that the tensile strength of a healing wound in guinea-pig suffering from partial deficiency of ascorbic acid is considerably less than in normal animals.

Summary. 1. Rabbits previously bruised healed more rapidly following injections of trypsin and SK-SD than did their untreated counterparts. 2. Injections of enzyme preparations into tissue remote from the bruised areas did not result in accelerated healing. 3. Combination of Vit. C and trypsin was superior to trypsin alone in promoting tissue repair.

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Effect of 6-Dimethylaminopurine-3-Amino-D-Ribose on Adenosine Triphosphate Formation in Yeast.* (24178)

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It has been demonstrated that 6-dimethylamino-purine-3-amino-d-ribose (Aminonucleoside) induces the nephrotic syndrome in rats (1,2). In an effort to obtain evidence of the mechanism of production of this experimental nephrosis, the effect of this compound on adenosine metabolism has been investigated. This study was prompted by reports which indicate that Aminonucleoside may interfere with some phase of purine metabolism. Agosin and Von Brand(3) and Hewitt, Gumble, Wallace and Williams(4,5) demonstrated that the trypanosomacidal action of the drug in infected mice could be blocked by simultaneous administration of various substituted purines. These observations, and similarity in chemical configuration of adenosine and Aminonucleoside, suggested the possibility of competitive inhibitory effect of Aminonucleoside upon adenosine metabolism. To test this hypothesis, an *in vitro* system known to metabolize adenosine was sought. Crude yeast fractions

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possess an enzyme, designated by Sable(6) as adenosine phosphokinase. In such fractions, a net production of adenosine triphosphate (ATP) from adenosine occurs.† Kornberg and Pricer(7) partially purified the yeast enzyme. Although 17 nucleosides were tested, substrate specificity was shown only for adenosine and 2-amino-adenosine. Aminonucleoside was not included in compounds studied. Caputto(8) subsequently found adenosine phosphokinase in animal tissue, namely kidney and liver, as well as in yeast. Studies of the animal enzyme indicated that adenosine in the presence of ATP was metabolized to adenosine-5¹-phosphate (AMP). Similar findings were obtained with yeast enzyme(7). It was therefore concluded that in crude yeast systems where ATP is formed from adenosine, the synthesis is stepwise from AMP. The subsequent steps involve formation of adenosine-diphosphate (ADP) by myokinase and of ATP by such enzymes as pyruvate phosphokinase. Our work is concerned with studies in a crude yeast system where the effect of Aminonucleoside on conversion of adenosine to ATP was investigated.

Materials and methods. Materials: ATP, triphosphopyridine nucleotide (TPN), glucose-6-phosphate dehydrogenase (Zwischenferment) and hexokinase were obtained from Sigma Chemical Co. The adenosine used was the product of Schwarz Laboratories. Dried brewer's yeast was supplied by Sigma Chemical Co. (Lot No. 24-605). We are indebted to Lederle Laboratories for 6-dimethyl-aminopurine-3-amino-d-ribose (Aminonucleoside). *Methods*: Incubations were carried out in Erlenmeyer flasks. The incubation mixture was as follows: 2.5 g of yeast and 0.05 ml toluene in 10 ml 0.1 *M* Na_2HPO_4 buffer, pH 7.0. By means of an electromagnetic device, stirring was continued throughout incubation period. Temperature was maintained at 25°C. Adenosine, the substrate, was added at final concentration of 37.0 μM/ml. Aminonucleoside, in concentrations of 37.0 to 185 μM/ml was tested in the absence and presence of adenosine. Aliquots of 0.1 ml were taken at 0 time and at 10 minute intervals over 60-120 minute incubation. Deproteinization of aliquots was accomplished in 10 ml of 10% trichloracetic acid (TCA). 1.0 ml aliquots of the centrifuged supernatant were then analyzed for inorganic phosphate(9) and acid-labile phosphate. Acid-labile phosphate was determined as that phosphate liberated in 10 minutes at 100°C in 1 *N* H_2SO_4. Measurements of ATP and other phosphate esters formed during incubation were obtained as follows: Reaction mixtures were cooled to 2°C in ice bath. 0.6 ml of 60% perchloric acid was added. The mixture was then filtered and precipitate washed with 2 ml portions of 3% perchloric acid. To combined filtrates were added 4 volumes of 95% ethanol. The precipitate was collected by centrifugation and taken up in 2.0 ml H_2O. The pH was then adjusted to 7.0. This material was then assayed spectrophotometrically for glucose-6-phosphate by reduction of TPN in the presence of Zwischenferment and hexokinase. Absence of glucose in each sample was ascertained by Somogyi-Nelson method(10). After completion of reaction, glucose was added and ATP determined, as described by Kornberg (11). Total phosphate was determined after ashing in 10 *N* H_2SO_4. Paper chromatographic separation of nucleotides and sugar phosphate esters was carried out on these extracts according to the methods of Krebs and Hems(12) and Bandursky and Axelrod(13), respectively.

Results. I. Inorganic phosphate uptake and acid labile phosphate formation. *Yeast and yeast plus adenosine.* Under incubation conditions described, in absence of added substrate a disappearance of inorganic phosphate (Pi) from the medium is noted. Maximum disappearance occurred after 60 minutes. During this period 14.9 μM/ml of acid-labile phosphate (Po) was formed. On addition of the substrate, adenosine, maximal uptake of Pi was increased and Po production was more than doubled (Table I, Exp. 1). Fig. 1 illustrates the time course of these changes.

Yeast plus Aminonucleoside. In contrast to the effect produced by adenosine, addition of equimolar amounts of Aminonucleoside as

† Personal communication from Sigma Chemical Co.

substrate produced only a small increase in Pi uptake over that noted in the absence of substrate. Further, an actual decrease in the amount of Po formed was noted. (Table I, Exp. II).

Yeast, adenosine and Aminonucleoside. In the presence of adenosine, addition of Aminonucleoside exerted an inhibitory effect on Pi uptake and Po formation, the magnitude of which was dependent upon concentration of Aminonucleoside (Table I, Exp. III). When Aminonucleoside and adenosine were present in equal concentrations, maximal amount of Po formed was equal to that formed in the absence of the Aminonucleoside, although there was a significant time lag (Fig. 1). When concentration of Aminonucleoside was increased to twice that of adenosine, Po formation declined from a control value of 31.0 μM/ml to 6.8 μM/ml and Pi uptake decreased in a like manner. Inhibition of Po formation became complete as concentration of Aminonucleoside was increased to 5 times that of adenosine.

II. ATP Production. To identify the Po or acid-labile phosphate compounds formed in the yeast incubation mixture, the perchloric acid-ethanol procedure was carried out on mixtures indicated in Table II. These extracts were then analyzed by paper chromatography(12). ATP was easily seen in all reaction mixtures except that containing 0.1875 *M* Aminonucleoside, that is, a 5:1 ratio of Aminonucleoside to adenosine. The adenosine incubation mixture showed the largest ATP spot. No other nucleotides were identified. To obtain more quantitative data, ATP was enzymatically assayed (Table II). These results are in accord with labile phosphate formation experiments in the following respects: (1) addition of adenosine to yeast resulted in a significant increase in ATP production; (2) a very small increase in ATP was noted upon addition of Aminonucleoside to yeast; and (3) in the presence of adenosine and high concentrations of Aminonucleoside, virtually complete inhibition of ATP production was observed.

III. Phosphate analysis. A representative group of ethanol precipitates was chosen for complete phosphate analysis (Table III). For each μmole of ATP one should find 2 μmoles of acid-labile phosphate; however, it was observed that in each precipitate this ratio was greater than 2:1. (The exception of

TABLE I. Effect of Aminonucleoside on Maximal Acid Labile Phosphate Production and Inorganic Phosphate Uptake.

Exp.		Change in acid labile phosphate (Po), μM/ml	Change in inorganic phosphate (Pi), μM/ml
I	Control	+14.9 (2)*	−70.4 (2)
	Exp.	+40.7 (2)	−81.5 (2)
II	Control	+13.2 (2)	−58.1 (2)
	Exp.	+ 9.4 (2)	−64.0 (2)
III	Control	+31.0 (6)	−86.3 (6)
	Exp. 1	+34.5 (2)	−82.6 (2)
	2	+ 6.8 (2)	−27.4 (2)
	3	− 5.5 (2)	+ 1.3 (2)

Exp. I Control: Yeast only
Exp.: " + adenosine 0.0375 M

Exp. II Control: " only
Exp.: " + aminonucleoside 0.0375 M

Exp. III Control: Yeast + adenosine 0.0375 M
Exp. 1: *Idem* + aminonucleoside 0.0375 M
2: " + " 0.0750 M
3: " + " 0.1875 M

All mixtures contained phosphate buffer, toluene and yeast as described. Values represent results obtained at point of maximal Pi uptake and Po formation. Control values were obtained simultaneously with exp. values.

* No. of experiments.

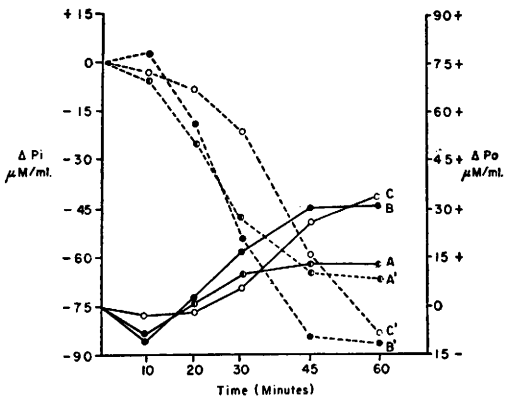

FIG. 1. All experimental mixtures contained phosphate buffer, toluene, and yeast as described above. - - - - represents uptake of inorganic phosphate and ——— formation of acid labile phosphate. A and A′ show results of incubating yeast alone; B and B′, the results when 0.0375 M adenosine is added and C and C′, the findings when 0.0375 M adenosine plus 0.0375 M aminonucleoside is incubated.

TABLE II. ATP Production.

Incubation mixtures	Yeast only	Yeast + Adenosine, .0375 M	Yeast + Nucleoside, .0375 M	Yeast + Adenosine, .0375 M; nucleoside, .1875 M
ATP, μM/g yeast	4.2 (4)*	12.7 (4)	5.5 (3)	1.7 (3)

* No. of experiments.

precipitate 1 is unexplained). Partial hydrolysis of more stable phosphate esters could account for this. Consequently, the materials were analyzed chromatographically for other acid-hydrolyzable phosphate compounds(13). These analyses revealed the presence of 2-phosphoglyceric acid, 3-phosphoglyceric acid, fructose-1-6 diphosphate, and glucose-6-phosphate in each of the precipitates except number IV. The latter contained only inorganic phosphate and glucose-6-phosphate, both of which appeared to be present in larger quantities than in the other precipitates. Glucose-6-phosphate assayed enzymatically substantiated the chromatographic observation. The quantitative data are presented in Table III.

Discussion. An inhibitory effect of Aminonucleoside on ATP production from adenosine has been demonstrated in a crude yeast system. In attempting to evaluate the mechanism involved in this inhibition, it must first be noted that formation of ATP in this system is not limited to a single reaction but may proceed as follows(7):

$$\text{Adenosine} + \text{ATP} \xleftarrow{\text{adenosine phosphokinase}} \text{AMP} + \text{ADP}$$

$$\text{ATP} + \text{AMP} \xleftarrow{\text{myokinase}} 2\ \text{ADP}$$

$$3\ \text{ADP} + 3\ \text{phosphopyruvate} \xrightarrow{\text{pyruvate phosphokinase}} 3\ \text{pyruvate} + 3\ \text{ATP}$$

It is possible then, that inhibition may be at one of several levels. The most likely site, however, would be at the level of adenosine phosphokinase. Direct evidence bearing on this question would require study of the purified enzyme. In this way a competitive type of inhibition might well be demonstrated.

Our data indicate that a significant ATP inhibition occurs in the presence of high concentration of Aminonucleoside. This is confirmed not only by direct measurements of the nucleotides but by associated findings of measurable levels of glucose-6-phosphate in the absence of hexose-di-phosphate and phosphoglyceric acids. This is in sharp contrast to the uninhibited adenosine metabolizing system in which the latter compounds are easily demonstrable. These observations are compatible with formation of glucose-6-phosphate from glycogen, a reaction which does not require ATP. On the other hand, ATP requiring reactions are depressed and this is reflected in the absence of other phosphorylated sugars.

In appraising the relationship between ability of Aminonucleoside to produce the nephrotic syndrome and its inhibitory effect on ATP formation, the following may be pertinent: (1) The earliest lesion observed in animals treated with this nucleoside, which precedes appearance of proteinuria, is the development of glomerular-podocyte damage as noted by Harkin and Recant in electron microscope studies (unpublished) and (2) evidence to date tends to implicate the glomerulus in the etiology of proteinuria in nephrosis (14). It seems reasonable to conclude that the Aminonucleoside may induce proteinuria and initiate the nephrotic syndrome by inter-

TABLE III. Phosphate* Analyses of Ethanol Precipitates.

Ppt. No.	Chemical analyses: Total Po_4	Inorganic Po_4	Acid-labile Po_4	Enzymatic analyses: ATP	Glucose-6 Po_4
I	18.3	.6	4.75	3.6	11.0
II	52.45	1.41	25.3	11.5	10.8
III	28.0	1.41	10.9	4.9	10.8
IV	19.0	6.50	1.87	0	13.4

Ppt. I Yeast only
II " + adenosine .0375 M
III " + aminonucleoside .0375 M
IV " + adenosine .0375 M and aminonucleoside .1875 M

* All data presented as μM/g yeast.

fering with production of high energy phosphate compounds in key cells within the glomerulus. This hypothesis raises a question concerning enzymatic activity and metabolic requirements of the normally functioning glomerulus. Experiments to explore this question are underway.

Summary. Production of acid-labile phosphate by crude brewer's yeast system, in the presence of adenosine and inorganic phosphate, has been demonstrated. This acid-labile phosphate has been identified as adenosine triphosphate. The nucleoside, 6-dimethylaminopurine-3-amino-d-riboside inhibits adenosine triphosphate formation in this system. The possible relationship of this inhibitory action to ability of the nucleoside to produce nephrosis in rats is discussed.

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Effects of 5-Flurouracil on Human Bronchogenic Carcinoma Grown in Hamster Cheek Pouch.* (24179)

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It has been shown that pyrimidine analogue 5-flurouracil has appreciable tumor-inhibitory activity against a variety of transplantable rat and mouse tumors(1). The work described here is a preliminary study of the efficacy of this drug on heterologously grown human bronchogenic carcinoma transplanted in the hamster cheek pouch.

Materials and methods. The tumor was the Skiff Bronchogenic Carcinoma obtained from Sloan-Kettering Institute for Cancer Research, N.Y. City. The host animal used was the golden hamster (*Mesocricetus auratus*), and site of tumor growth was the left cheek pouch. Experimental animals 40 to 80 g were obtained from National Pet Shops, St. Louis, Mo. Animals of both sexes were used. The tumor transplant was introduced into the cheek pouch under light ether anesthesia with a No. 14 trocar, by the method of Lutz, *et al.*(2) as modified by Toolan(3), Patterson, *et al.*(4) and Palm, *et al.* (unpublished). Microscopic examination of a hematoxylin eosin stained section at time of transplantation revealed that the tumor was composed of fairly uniform cells of moderate size with hyperchromic nuclei and fairly large amounts of deeply staining cytoplasm. The cells tended to be spindle-shaped and elongated in many areas. Only rare giant nuclei were present and multi-nucleated forms were not seen. A bronchogenic tumor in man with these morphologic characteristics would usually be classified as an undifferentiated carcinoma of the "large cell" type. The tumor specimen used for transplantation was harvested from the cheek pouch of a maintenance animal, in which it had grown for 14

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